Non-traditional risk factors predict coronary calcification in chronic kidney disease in a population-based cohort

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The increased burden of cardiovascular disease in chronic kidney disease cannot be explained by traditional risk factors alone. Here, we evaluated the impact of non-traditional factors on the association of chronic kidney disease with coronary artery calcification using logistic regression among 2672 Dallas Heart Study patients of whom 220 had chronic kidney disease. The prevalence of coronary calcification significantly increased across all chronic kidney disease stages and this remained independently associated with coronary calcification after adjusting for traditional factors. The calcium × phosphorus product, homocysteine, and osteoprotegerin each diminished the magnitude of association between kidney disease and coronary calcification. After adjustment for these, the association between kidney disease and coronary calcification was no longer significant with the effects most prominent in the stages 3–5 subgroup. Our study has identified three non-traditional independent predictors of coronary calcification that diminished the association between chronic kidney disease and coronary calcification. These factors may represent novel mechanistic links warranting further investigation.

Kidney International (2008) **73,** 615–621; doi:10.1038/sj.ki.5002716; published online 12 December 2007

KEYWORDS: coronary calcification; chronic kidney disease; risk factors

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Received 11 June 2007; revised 19 September 2007; accepted 9 October 2007; published online 12 December 2007

Excess cardiovascular morbidity and mortality are well-known complications of end-stage renal disease.[1,2] Recent studies have firmly established that pre-dialysis chronic kidney disease (CKD) is also independently associated with incident cardiovascular disease (CVD) and adverse cardiovascular outcomes.[3–7] Even subjects with mild reductions in glomerular filtration rate (GFR) of 60–89 ml per min per 1.73 m^2 demonstrate a 25% increased risk of developing CVD relative to those with a GFR > 90.[5] A portion of this elevated risk may be due to the increased burden of concomitant CVD risk factors prevalent in patients with CKD, such as hypertension, diabetes mellitus, and dyslipidemia, factors that are less well controlled among CKD vs non-CKD populations.[8] However, CKD remains a strong and independent predictor of CVD risk even after adjustment for these traditional factors,[3–7] suggesting that the association between the two disease states may be mediated by non-traditional CVD risk factors.

Evaluation of non-traditional factors may identify additional independent predictors of coronary atherosclerosis that may further attenuate the association between CKD and CVD and represent novel mechanistic links in the development of atherosclerotic disease among patients with CKD. We analyzed data from a large, multiethnic, probability-based community cohort to assess the degree to which traditional and non-traditional risk factors modify the association between CKD and CVD, using coronary artery calcification (CAC) as a surrogate marker of coronary atherosclerosis.[9–11]

RESULTS

Baseline characteristics

The cohort had a mean age of 45.2 ± 9.3 years. Among the 2672 participants, 45.0% were male and 48.8% were African American. Prevalences of hypertension, diabetes, and hypercholesterolemia were 32.0, 11.3, and 13.4%, respectively. Two hundred and fifty-two (9.4%) had CAC scores ⩾100 and 2420 (90.6%) had scores <100. CKD prevalence was 19.4% among those with CAC⩾100 and 7.1% with CAC<100 ($P<0.0001$).

Table 1 | Clinical and biochemical parameters by CKD stages[a]

Independent variables	No CKD (*n*=2452)	CKD stages 1–2 (*n*=170)	CKD stages 3–5 (*n*=50)	*P*-value
Traditional risk factors				
Age (years)	44.9 ± 9.3	47.3 ± 9.1	52.5 ± 7.7	<0.0001
Male sex (%)	44.4	54.7	44.0	0.03
African-American race (%)	47.3	67.7	62.0	<0.0001
Family history of myocardial infarction (%)	32.2	37.1	46.0	0.06
History of smoking (%)	28.2	32.9	38.0	0.14
Diabetes mellitus (%)	9.3	34.7	30.0	<0.0001
Hypertension (%)	29.7	55.9	64.0	<0.0001
Hypercholesterolemia (%)	13.2	14.8	20.0	0.32
Non-traditional risk factors				
Homocysteine ($\mu mol\,l^{-1}$)	8.8 ± 3.8	10.1 ± 13.3	15.5 ± 7.6	<0.0001
Calcium (mg per 100 ml)	9.3 ± 0.4	9.3 ± 0.4	9.4 ± 0.6	0.0009
Phosphorus (mg per 100 ml)	3.2 ± 0.6	3.2 ± 0.6	3.7 ± 1.0	<0.0001
Calcium × phosphorus product (mg^2 per $100\,ml^2$)	29.6 ± 5.4	30.1 ± 6.1	35.2 ± 10.4	<0.0001
C-reactive protein ($mg\,l^{-1}$)	2.7 (1.1, 6.3)	3.6 (1.6, 7.8)	3.7 (1.8, 9.7)	0.003
Lipoprotein (a) ($nmol\,l^{-1}$)	47.2 (17.7, 106.8)	54.9 (24.1, 105.0)	62.1 (25.4, 132.1)	0.16
Small LDL subclass (mg per 100 ml cholesterol)	5.6 (0.0, 27.2)	9.8 (0.0, 39.0)	0.0 (0.0, 17.2)	0.02
Osteoprotegerin ($pg\,ml^{-1}$)	1193.5 (870.5, 1582.1)	1247.2 (943.6, 1892.0)	1420.2 (1015.9, 2272.0)	0.0004

CKD, chronic kidney disease; LDL, low-density lipoprotein.
[a]Continuous variables with normal distribution are reported as mean ± s.d. Continuous variables with non-normal distribution are reported as median (interquartile range). Categorical variables are reported as percentages.

There were 220 patients with CKD, of whom 170 (77.3%) had CKD stages 1–2 and 50 (22.7%) had CKD stages 3–5. The prevalence of CAC ⩾ 100 increased significantly across CKD stages, from 8.3% in non-CKD patients to 16.5% in patients with CKD stages 1–2 and 42.0% among those with CKD stages 3–5 (trend *P*-value <0.0001). CKD patients were older, and a greater proportion was African American and had a history of diabetes mellitus or hypertension as compared to those without CKD (Table 1). Across stages of renal dysfunction, significant increases were observed for levels of calcium, phosphorus, calcium × phosphorous product (CPP), homocysteine (HCY), C-reactive protein (CRP), and osteoprotegerin (OPG) (Table 1).

Associations between risk factors and CAC

All traditional and non-traditional factors tested in univariable models, with the exception of lipoprotein (a) (Lp (a)) and small low-density lipoprotein (LDL) subclass, were significantly and positively associated with CAC ⩾ 100 (Table 2). Prevalent CKD was also significantly associated with CAC ⩾ 100 (odds ratio (OR) 3.18, 95% confidence interval (95% CI) 2.24–4.50).

Although addition of African-American race and traditional risk factors to the logistic regression model diminished the association between CKD and CAC ⩾ 100, CKD remained significantly associated with CAC ⩾ 100 (OR 1.76, 95% CI 1.16–2.68) (Table 3). Further attenuation was observed in the association between CKD and CAC after adjustment for calcium, phosphorus, CPP, HCY, or OPG, whereas CRP did not markedly influence the association (Table 3). CPP attenuated the effect of CKD on CAC to a greater degree than phosphorus or calcium alone and so was included in the final multivariable model (Table 3). The association between CKD and CAC ⩾ 100 was no longer statistically significant once CPP, HCY, and OPG were added together to the final

Table 2 | Univariable logistic models for the associations between variables and CAC ⩾ 100

Variable	OR (95% CI)	*P*-value
Traditional		
Age (per year increase)	1.14 (1.12–1.16)	<0.0001
Male sex	2.38 (1.82–3.12)	<0.0001
African-American race	1.64 (1.26–2.14)	<0.0001
Family history of myocardial infarction	2.12 (1.63–2.75)	<0.0001
History of smoking	2.02 (1.55–2.64)	<0.0001
Diabetes mellitus	3.30 (2.42–4.51)	<0.0001
Hypertension	4.48 (3.41–5.88)	<0.0001
Hypercholesterolemia	3.24 (2.41–4.37)	<0.0001
Non-traditional		
Chronic kidney disease	3.18 (2.24–4.50)	<0.0001
Homocysteine ($\mu mol\,l^{-1}$)[a]	1.06 (1.03–1.08)	<0.0001
Calcium (mg per 100 ml)[a]	2.31 (1.62–3.30)	<0.0001
Phosphorus (mg per 100 ml)[a]	1.33 (1.08–1.65)	0.0092
Calcium × phosphorus product (mg^2 per $100\,ml^2$)[a]	1.05 (1.03–1.07)	<0.0001
Log C-reactive protein[a]	1.19 (1.06–1.33)	0.002
Lp (a) tertile 2 vs 1[b]	1.55 (1.03–2.33)	0.09
Lp (a) tertile 3 vs 1[b]	1.89 (0.55–6.54)	0.09
Small LDL tertile 2 vs 1[b]	1.21 (0.79–1.85)	0.37
Small LDL tertile 3 vs 1[b]	1.98 (0.67–5.85)	0.37
Log osteoprotegerin[a]	1.97 (1.56–2.49)	<0.0001

CAC, coronary artery calcification; CI, confidence interval; LDL, low-density lipoprotein; Lp (a), lipoprotein (a); OR, odds ratio.
[a]Per unit increase.
[b]Tertile 1 represents lowest tertile and tertile 3 represents highest tertile.

multivariable model containing the traditional risk factors (OR 1.41, 95% CI 0.91–2.18).

Stratification by CKD stages

Univariable and multivariable analyses were repeated after stratifying the population by CKD stages (Table 4). In the univariable analyses, CKD stages 1–2 and CKD stages 3–5 (vs no CKD) were both significantly associated with CAC $\geqslant$ 100, with a larger point estimate of association observed for CKD stages 3–5 (OR 8.02, 95% CI 4.49–14.32) as compared with CKD stages 1–2 (OR 2.19, 95% CI 1.42–3.36).

In multivariable analyses, the association between CKD stages 1–2 and CAC $\geqslant$ 100 was completely attenuated with the inclusion of African-American race and traditional risk factors, whereas only partial attenuation was observed for CKD stages 3–5, which remained an independent predictor of CAC (Table 4). Calcium, phosphorus, CPP, HCY, and OPG each separately further attenuated the association between CKD stages 3–5 and CAC $\geqslant$ 100, whereas CRP did not (Table 4). Similar to the non-stratified analysis (Table 3), CPP attenuated the effect of CKD on CAC to a greater degree than phosphorus or calcium alone and so was included in the final multivariable model (Table 4). After CPP, HCY, and OPG were entered together into the final multivariable model, the association between CKD stages 3–5 and CAC $>$ 100 was attenuated but remained statistically significant (OR 2.38, 95% CI 1.15–4.95).

Sensitivity analysis using a CAC cut-off of $\geqslant$30 instead of $\geqslant$100 for logistic models revealed similar results and did not change the main findings of this study. The overall accuracy of the logistic models using CAC $\geqslant$ 30 was also similar to the models using CAC $\geqslant$ 100 (c-statistic of 0.86 and 0.87, respectively). Sensitivity analysis performed excluding seven patients who responded 'yes' to the question 'do you have or have you ever had chronic kidney failure requiring dialysis' decreased the magnitude of association between any CKD and CAC and attenuated the association of CKD stages 3–5 with CAC once all non-traditional factors were entered into the final model (OR 1.88, 95% CI 0.85–4.14).

Table 3 | Multivariable logistic models for the association between CKD and CAC $\geqslant$ 100

Model	CKD OR (95% CI)	C-statistic
1. CKD alone	3.18 (2.24–4.50)	0.562
2. CKD+traditional factors[a]	1.76 (1.16–2.68)	0.867
3. Model 2+calcium	1.71 (1.12–2.60)	0.868
4. Model 2+phosphorus	1.67 (1.09–2.54)	0.869
5. Model 2+CPP	1.62 (1.06–2.48)	0.871
6. Model 2+homocysteine	1.56 (1.01–2.39)	0.867
7. Model 2+log osteoprotegerin	1.67 (1.10–2.55)	0.869
8. Model 2+log C-reactive protein	1.77 (1.16–2.70)	0.866
9. Model 2+CPP, HCY, log OPG	1.41 (0.91–2.18)	0.873

CAC, coronary artery calcification; CKD, chronic kidney disease; CI, confidence interval; CPP, calcium $\times$ phosphorous product; HCY, homocysteine; OR, odds ratio; OPG, osteoprotegerin.
[a]Covariates included were age, male gender, family history of myocardial infarct, history of smoking, diabetes mellitus, hypertension, and hypercholesterolemia, in addition to African-American race.

DISCUSSION

The principal new finding in this study is that the non-traditional risk factors CPP, OPG and HCY diminished the association between CKD and clinically relevant CAC. This finding suggests that pathways represented by these markers (such as vascular calcification, endothelial injury, and accelerated thrombosis) may contribute to the excess burden of coronary artery disease observed in patients with CKD. Furthermore, the attenuation of the association between CKD and CAC observed with these non-traditional factors appeared to be stronger at advanced stages of CKD vs earlier stages, whereas traditional risk factors appear to exert their effect at both CKD stages. This important new finding in a relatively young, multiethnic cohort extends previous findings from studies performed in older populations.[12–16] An alternative explanation is that our cohort is relatively young, and thus contributions from traditional risk factors may be of a lesser degree than in older CKD populations. The enhanced effect of non-traditional risk factors at more advanced (vs milder) stages of CKD is biologically plausible, as the metabolic and hemodynamic alterations associated with CKD progress as GFR declines and may provide the proper milieu for the development of accelerated atherosclerosis. Elevations in serum parathyroid hormone, for example, do not occur until GFR decreases below 70 ml per min per

Table 4 | Multivariable logistic models for CAC $\geqslant$ 100 stratified by CKD stages

Model	CKD stages 1–2[a]	CKD stages 3–5[a]	C-statistic
1. CKD alone	2.19 (1.42–3.36)	8.02 (4.49–14.32)	0.564
2. CKD+traditional factors[b]	1.16 (0.69–1.93)	4.31 (2.20–8.43)	0.870
3. Model 2+calcium	1.12 (0.67–1.89)	4.11 (2.10–8.05)	0.870
4. Model 2+phosphorus	1.16 (0.69–1.94)	3.60 (1.82–7.13)	0.870
5. Model 2+CPP	1.16 (0.69–1.94)	3.33 (1.68–6.62)	0.873
6. Model 2+homocysteine	1.14 (0.68–1.92)	3.12 (1.53–6.34)	0.869
7. Model 2+log osteoprotegerin	1.12 (0.67–1.87)	3.91 (1.99–7.70)	0.872
8. Model 2+log C-reactive protein	1.17 (0.70–1.95)	4.30 (2.20–8.42)	0.869
9. Model 2+CPP, HCY, log OPG	1.12 (0.67–1.89)	2.38 (1.15–4.95)	0.874

CAC, coronary artery calcification; CKD, chronic kidney disease; CPP, calcium $\times$ phosphorous product; HCY, homocysteine; OPG, osteoprotegerin.
[a]Odds ratios are for CKD stages 1–2 vs no CKD and CKD stages 3–5 vs no CKD.
[b]Covariates included were age, male gender, family history of myocardial infarct, history of smoking, diabetes mellitus, hypertension, and hypercholesterolemia, in addition to African-American race.

1.73m^2,[17] and mean serum phosphorus levels do not increase rapidly until GFR declines to below 40 ml per min per 1.73 m^2.[18]

In contrast to most previous studies that involved CKD patients on chronic dialysis, our study evaluating the relationship between calcium, phosphorus, CPP, and CAC involved patients with earlier stages of CKD. Only 7 of 2672 had been or were currently on hemodialysis, and the exclusion of those patients did not alter the results considerably except that, as expected, it decreased the magnitude of the association between CKD and CAC.

Although elevated CPP has been reported to be an independent predictor of mortality in dialysis patients,[19] studies evaluating the relationship between various indices of calcium/phosphorus homeostasis and CAC in pre-dialysis CKD patients have generated inconsistent results.[12–16,20,21] In fact, the National Kidney Foundation guidelines regarding management of calcium/phosphorus homeostasis, such as desired parathyroid hormone levels, are opinion based rather than evidence based for pre-dialysis stages 3 and 4 CKD.[17] Two earlier studies reported a positive association between serum phosphorus levels and CAC or CAC progression in such patients.[16,20] A graded independent relationship between higher levels of serum phosphorus and risk of death and cardiovascular events was reported in patients with previous myocardial infarct, most of whom had phosphorus levels within the normal range.[22] Our results extend these observations as we found that calcium, phosphorus, and CPP were all positively associated with CAC, and each diminished the association between CKD and CAC in multivariable models, with CPP causing the greatest attenuation. The c-statistics of the multivariable models for CAC were the same when either calcium or phosphorus was included in separate models (both 0.87), indicating that both variables are similarly contributing to the model fit. More importantly, this effect was observed in a population with mean CPP levels of 35.2 mg^2 per 100 ml^2, well below the level of 55 mg^2 per 100 ml^2 previously associated with increased cardiovascular risk in chronic dialysis patients.[19] This suggests that even the modest perturbations in calcium/phosphorus metabolism observed in patients with earlier stages of CKD may contribute to coronary calcification. Large prospective interventional trials are needed to investigate whether more aggressive control of CPP in the pre-dialysis phase of CKD may decrease future risk of CVD.

We found that HCY had a modest impact on the association between CKD stages 3–5 and CAC. Although levels of HCY are elevated among pre-dialysis CKD vs non-CKD patients,[23,24] less is known regarding the CVD risk associated with hyperhomocysteinemia in such subjects. Five previous studies examining the relationship between CKD and CAC did not include HCY as a covariate.[12–16] Two other studies, however, demonstrated an association between HCY and cardiovascular events in either pre-dialysis CKD or kidney transplant recipients.[25,26] Our results are consistent with these latter observations and suggest that the increased CVD risk conferred by HCY in dialysis patients[27,28] may begin in the pre-dialysis disease phase and may be due to its contribution to coronary atherosclerosis burden.[29,30] It should be noted, unfortunately, that the excess risk attributable to HCY in patients with advanced CKD may not be modifiable with folic acid and vitamin B therapy.[31]

OPG diminished the association between CKD stages 3–5 and CAC scores $\geqslant$100, suggesting that OPG may contribute to the increased risk of CVD observed in these patients. OPG functions as a regulator of bone resorption by inhibiting osteoclastogenesis.[32] The mechanisms responsible for the observed association between OPG and arterial calcification remain poorly understood and appear paradoxical. For example, OPG-deficient mice develop widespread arterial calcifications,[33] whereas serum OPG levels are higher in subjects with greater amounts of coronary calcification.[34–37] In fact, a U-shaped association between OPG and all-cause mortality has been described in hemodialysis patients in the presence of inflammation.[38] One potential hypothesis is that OPG expression increases in the setting of pre-existing calcification and acts in a compensatory manner to minimize further calcification. Another explanation is that OPG directly contributes to vascular calcification by promoting a low bone turnover state.[39] In this context, the ability of bone to take up a mineral load is diminished,[40] which might favor extra-skeletal calcification. Studies involving CKD patients, however, have been limited either to those with stage 5 CKD before and after dialysis initiation or to renal transplant recipients. Our findings extend these observations by suggesting that OPG may promote coronary calcification in earlier CKD stages and could possibly serve as a biomarker to assess CVD risk among pre-dialysis CKD patients.

Despite the established association between various non-traditional risk factors (such as anemia, measures of inflammation, and calcium/phosphorus homeostasis) and poor cardiovascular outcomes in dialysis patients, there are few data regarding such factors in patients with pre-dialysis CKD. Our results suggest that novel pathways represented by HCY, CPP, and OPG may promote CVD in patients with moderate to severe pre-dialysis CKD. If validated using other measures of atherosclerosis and in cohort studies with adequate numbers of clinical events, these findings could have important clinical and therapeutic implications. These novel factors may assist in risk assessment of patients with CKD, to determine which are at highest risk for adverse CV events and require the most aggressive preventive therapies. Alternatively, if these factors are causally related to CVD in patients with CKD, they may serve as therapeutic targets. For example, the CPP target for treatment may need to be lowered in stages 3–5 pre-dialysis CKD.[18] Therapies targeting receptor activator of nuclear factor-κB (RANK) and RANK ligand may merit investigation as targets for CVD prevention in patients with CKD.[38] Larger, prospective studies are needed to determine whether these non-traditional factors play a critical role in development of CVD, and interventional trials are required to investigate whether modification

of these factors may decrease cardiovascular morbidity and mortality.

Our study is not without limitation and due to its cross-sectional nature cannot imply a causal relationship between non-traditional risk factors and CAC. Biochemical parameters, including urine microalbumin, were measured once and might not reflect actual levels over time. In addition, we were unable to adjust for certain confounders, such as intact parathyroid hormone, anemia, and fibrinogen, that are associated with either CAC or CVD risk among CKD patients. Although we controlled for diabetes in all of the multivariable models, diabetics comprised only 30% of the subgroup with CKD, and therefore these findings should be confirmed in populations with a greater proportion of diabetic nephropathy. We evaluated a limited number of non-traditional cardiovascular risk markers that were available in the Dallas Heart Study database and did not evaluate several others, such as asymmetric dimethyl arginine (ADMA)[41] and fetuin A,[42] that have shown promise in patients with CKD. Even though using a CAC score $\geqslant 100$ as our main outcome measure makes our findings more clinically relevant, coronary calcification is only a surrogate marker of cardiovascular outcomes among patients with preserved renal function.[9–11] Less is known regarding the relationship between CAC and outcomes in CKD, although CAC scores were associated with clinically evident coronary artery disease among pre-dialysis CKD patients.[12]

Conclusions

We found that non-traditional cardiovascular risk factors diminished the association between clinically relevant CAC and CKD. This effect was strongest and most consistent in those with more advanced CKD. Non-traditional factors such as increased CPP, HCY, and OPG are more prevalent in CKD vs non-CKD populations and might partially explain the excessive cardiovascular morbidity and mortality in CKD patients. Future prospective studies are necessary to investigate the potential mechanistic relationships between non-traditional risk factors and CKD and to determine whether modifying such risk factors will improve outcomes.

MATERIALS AND METHODS

Study population

The Dallas Heart Study is a cross-sectional cohort study comprising a multiethnic, population-based, probability sample of Dallas County residents aged 30–65 years. The study design has been described previously.[43] After an initial in-home visit to obtain demographic and health-related data ($n = 6101$), a second in-home visit was performed where fasting venous blood and urine samples were collected ($n = 3557$), and a third visit to University of Texas Southwestern Medical Center where comprehensive imaging studies were obtained, including electron beam computed tomography (EBCT) scanning of the coronary arteries ($n = 2971$). Our analyses were restricted to the 2672 patients in whom there were sufficient data for estimations of GFR, urine microalbumin, and EBCT assessment of CAC.

Evaluation of kidney function

The urinary albumin/creatinine ratio (ACR) was calculated using a first morning void sample. GFR (ml per min per 1.73 m^2 body surface area) was estimated with the four-variable Modification of Diet in Renal Disease (MDRD) Study formula using the serum creatinine measurement: $GFR = 186 \times (\text{serum creatinine}^{-1.154}) \times (\text{age}^{-0.203}) \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$.[44] Using the National Kidney Foundation guidelines, CKD stages 1–2 were defined as an albumin/creatinine ratio ($mg\,g^{-1}$) $\geqslant 17$ in men and $\geqslant 25$ in women and a GFR $\geqslant 60$ ml per min per 1.73 m^2 body surface area, and CKD stages 3–5 were defined as an estimated GFR < 60.[45]

Evaluation of CAC

EBCT measurements were made at 80% of the RR interval using an Imatron 150 XP (Imatron Inc., San Francisco, CA, USA), 30 cm field of view, 512 matrix with sharp kernel reconstruction, and scores were expressed in Agatston units.[46] Calcium scoring followed the Multi Ethnic Study of Atherosclerosis (MESA) protocol.[47] The mean of two consecutive scans was used as the final EBCT score except in subjects in whom only one scan was obtained. A CAC score of $\geqslant 100$ was used to define clinically relevant CAC, as this cut-off corresponds to moderate to high 10-year risk of cardiovascular events.[9,48–52]

Clinical covariates

Race was dichotomized as African American or non-African American according to the subject's self-report. Five consecutive blood pressure (BP) readings were recorded at 1-min intervals, and the average of the third, fourth, and fifth measurements was used for analysis. Hypertension was defined as self-reported diagnosis or treatment for hypertension or a systolic BP $\geqslant 140$ mm Hg or a diastolic BP $\geqslant 90$ mm Hg. Diabetes definition was based on a self-report coupled with use of insulin or oral hypoglycemic medication; a fasting glucose $\geqslant 126$ mg per 100 ml or a non-fasting glucose $\geqslant$ 200 mg per 100 ml. Hypercholesterolemia was defined as a fasting calculated LDL cholesterol $\geqslant 160$ mg per 100 ml, direct LDL $\geqslant$ 160 mg per 100 ml on a non-fasting sample, total cholesterol $\geqslant$ 240 mg per 100 ml, or the use of statin medication. Family history of heart attack was self-reported and defined as history of heart attack in a first-degree relative (mother, father, sibling, or child). Smoking was defined as cigarette use within the previous 30 days and a lifetime history of having smoked $\geqslant 100$ cigarettes.

Blood samples were maintained at 4 °C until the plasma separated and placed in aliquots that were stored at −80 °C. Plasma Lp (a) was measured by a sandwich ELISA (enzyme-linked immunosorbent assay) that is insensitive to apolipoprotein(a) isoform size.[53] Lipoprotein subclass profiles were measured on plasma samples by a commercially available proton NMR (nuclear magnetic resonance) spectroscopic assay (LipoScience, Raleigh, NC, USA) as described previously.[54,55] The NMR method provides a direct measure of a given lipoprotein subclass. The small LDL subclass includes those particles ranging in size from 18.3 to 19.7 nm with a concentration reported in mass units of cholesterol (mg per 100 ml cholesterol). OPG measurements were performed in duplicate on thawed samples by an enzyme-linked immunoassay (R&D systems, Minneapolis, MN, USA).[56] The sensitivity of this assay, defined as the mean ± 3 s.d. of the zero standard, was calculated to be 15 $pg\,ml^{-1}$. CRP measurements were performed using a commercially available high-sensitivity assay (Roche Diagnostics, Indianapolis, IN, USA).[57] HCY was measured by LipoScience laboratory using chemiluminescence immunoassay

(DPC Immulite, Holliston, MA, USA). CPP was defined as the product of the serum calcium concentration and the serum phosphorus concentration in mg^2 per 100 ml^2.

Statistical analysis

Categorical data are reported as percentages and continuous data as means ± s.d. or medians with interquartile ranges. Comparisons were made across three groups based on CKD stages: no CKD, CKD stages 1–2, and CKD stages 3–5. Variables were compared between groups using the χ^2 test for categorical data. For continuous variables, one-way analysis of variance was used to compare those with Gaussian distributions, and Kruskal–Wallis test was used for those with non-Gaussian distributions.

Two sets of logistic regression models were constructed with CAC $\geqslant$ 100 as the dependent variable. In the first set, prevalent CKD was the main independent variable and was dichotomized as CKD presence vs no CKD. In the second set, CKD (as the main independent variable) was trichotomized into no CKD, CKD stages 1–2, and CKD stages 3–5, with no CKD as the referent group. Associations between the following variables and CAC $\geqslant$ 100 were tested in univariable logistic regression: African-American race and traditional risk factors, which included age, male sex, family history of myocardial infarction (MI), history of smoking, hypertension, diabetes mellitus, and hypercholesterolemia; and non-traditional factors, which included calcium, phosphorus, CPP, HCY, CRP, Lp (a), small LDL, and OPG. Owing to non-Gaussian distributions, OPG and CRP were log-transformed, whereas small LDL and Lp (a) were divided into tertiles. Only those covariates that had a *P*-value <0.05 in univariable models were tested as candidate variables in the multivariable logistic regression models. Calcium, phosphorus, and CPP were separately tested in multivariable models and were not included in the same models concurrently due to concerns for multicollinearity.

To test that choosing a higher but more specific CAC cut-off of $\geqslant$100 does not miss a number of subjects at considerable risk, we performed a sensitivity analysis using a CAC cut-off of $\geqslant$30 for logistic models. All statistical tests were two-sided, conducted at the standard significance level of 0.05 and reported using *P*-values and/or 95% CIs. All analyses were performed using SAS Enterprise Guide Version 3.0 and SAS software Version 9.1 (Cary, NC, USA).

DISCLOSURE

The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs.

ACKNOWLEDGMENTS

The Dallas Heart Study was supported by a grant from the Donald W Reynolds Foundation and by USPHS GCRC Grant no. M01-RR00633 from NIH/NCRR-CR. This work was also supported by the University of Texas Southwestern Medical Center O'Brien Kidney Research Core Center (P30DK079328). Parts of this data were presented in abstract form at the American Heart Association Scientific Sessions Annual Meeting in Chicago, Illinois, 12–15 November 2006.

REFERENCES

1. United States Renal Data System (USRDS). United States Renal Data System (USRDS) 2000 Annual Data Report: atlas of end-stage renal disease in the United States, Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2000.
2. Foley RN. Clinical epidemiology of cardiac disease in dialysis patients: left ventricular hypertrophy, ischemic heart disease, and cardiac failure. *Semin Dial* 2003; **16**: 111–117.
3. Anavekar NS, McMurray JV, Velazquez EJ *et al.* Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004; **351**: 1285–1295.
4. Go AS, Chertow GM, Fan D *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296–1305.
5. Manjunath G, Tighiouart H, Ibrahim H *et al.* Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol* 2003; **41**: 47–55.
6. Manjunath G, Tighiouart H, Coresh J *et al.* Level of kidney function as a risk factor for cardiovascular outcomes in the elderly. *Kidney Int* 2003; **63**: 1121–1129.
7. Muntner P, He J, Hamm L *et al.* Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. *J Am Soc Nephrol* 2002; **13**: 745–753.
8. Parikh NI, Hwang S, Larson MG *et al.* Cardiovascular disease risk factors in chronic kidney disease. *Arch Intern Med* 2006; **166**: 1884–1891.
9. Arad Y, Spadaro LA, Goodman K *et al.* Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol* 2000; **36**: 1253–1260.
10. Kondos GT, Hoff JA, Sevrukov A *et al.* Electron-beam tomography coronary artery calcium and cardiac events. A 37-month follow-up of 5635 initially asymptomatic low-to-intermediate risk adults. *Circulation* 2003; **107**: 2571–2576.
11. Rumberger JA, Simons B, Fitzpatrick LA *et al.* Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. *Circulation* 1995; **92**: 2157–2162.
12. Dellegrottaglie S, Saran R, Gillespie B *et al.* Prevalence and predictors of cardiovascular calcium in chronic kidney disease (from the prospective longitudinal RRI-CKD study). *Am J Cardiol* 2006; **98**: 571–576.
13. Mehrotra R, Budoff M, Christenson P *et al.* Determinants of coronary artery calcification in diabetics with and without nephropathy. *Kidney Int* 2004; **66**: 2022–2031.
14. Merjanian R, Budoff M, Adler S *et al.* Coronary artery, aortic wall, and valvular calcification in nondialyzed individuals with type 2 diabetes and renal disease. *Kidney Int* 2003; **64**: 263–271.
15. Qunibi WY, Abouzahr F, Mizani MR *et al.* Cardiovascular calcification in Hispanic Americans (HA) with chronic kidney disease (CKD) due to type 2 diabetes. *Kidney Int* 2005; **68**: 271–277.
16. Tomiyama C, Higa A, Dalboni MA *et al.* The impact of traditional and non-traditional risk factors on coronary calcification in pre-dialysis patients. *Nephrol Dial Transplant* 2006; **21**: 2464–2471.
17. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Kidney disease outcome quality initiative. *Am J Kidney Dis* 2002; **39**: S1–S246.
18. Kestenbaum B, Sampson JN, Rudser KD *et al.* Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005; **16**: 520–528.
19. Block GA, Klassen PS, Lazarus JM *et al.* Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 2004; **15**: 2208–2218.
20. Russo D, Corrao S, Miranda I *et al.* Progression of coronary artery calcification in predialysis patients. *Am J Nephrol* 2007; **27**: 152–158.
21. Russo D, Palmiero G, De Blasio A *et al.* Coronary artery calcification in patients with CRF not undergoing dialysis. *Am J Kidney Dis* 2004; **44**: 1024–1030.
22. Tonelli M, Sacks F, Pfeffer M *et al.* Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation* 2005; **112**: 2627–2633.
23. Bostom AG, Shemin D, Lapane KL *et al.* Hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein (a) excess in maintenance dialysis patients: a matched case-control study. *Atherosclerosis* 1996; **125**: 91–101.
24. Muntner P, Hamm LL, Kusek JW *et al.* The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med* 2004; **140**: 9–17.
25. Jungers P, Chauveau P, Blandin O *et al.* Hyperhomocysteinemia is associated with atherosclerotic occlusive arterial accidents in predialysis chronic renal failure patients. *Miner Electrolyte Metab* 1997; **23**: 170–173.
26. Massy ZA, Chadefaux-Vekemans B, Chevalier A *et al.* Hyperhomocysteinaemia: a significant risk factor for cardiovascular disease in renal transplant recipients. *Nephrol Dial Transplant* 1994; **9**: 1103–1108.

27. Buccianti G, Baragetti I, Bamonti F *et al.* Plasma homocysteine levels and cardiovascular mortality in patients with end-stage renal disease. *J Nephrol* 2004; **17**: 405–410.
28. Moustapha A, Naso A, Nahlawi M *et al.* Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 1998; **97**: 138–141.
29. Chao CL, Tsai HH, Lee CM. The graded effect of hyperhomocysteinemia on the severity and extent of coronary atherosclerosis. *Atherosclerosis* 1999; **147**: 379–386.
30. Rasouli ML, Nasir K, Blumenthal RS *et al.* Plasma homocysteine predicts progression of atherosclerosis. *Atherosclerosis* 2005; **181**: 159–165.
31. Jamison RL, Hartigan P, Kaufman JS *et al.* Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; **298**: 1163–1170.
32. Simonet WS, Lacey DL, Dunstan CR *et al.* Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309–319.
33. Bucay N, Sarosi I, Dunstan CR *et al.* Osteoprotegerin-deficient mice develop early-onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260–1268.
34. Abedin M, Omland T, Ueland T *et al.* Relation of osteoprotegerin to coronary calcium and aortic plaque (from the Dallas Heart Study). *Am J Cardiol* 2007; **99**: 513–518.
35. Mazzaferro S, Pasquali M, Pugliese F *et al.* Serum levels of calcification inhibition proteins and coronary artery calcium score: comparison between transplantation and dialysis. *Am J Nephrol* 2007; **27**: 75–83.
36. Nitta K, Akiba T, Uchida K *et al.* The progression of vascular calcification and serum osteoprotegerin levels in patients on long-term hemodialysis. *Am J Kidney Dis* 2003; **42**: 303–309.
37. Jono S, Ikari Y, Shioi A *et al.* Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. *Circulation* 2002; **106**: 1192–1194.
38. Morena M, Terrier N, Jaussent I *et al.* Plasma osteoprotegerin is associated with mortality in hemodialysis patients. *J Am Soc Nephrol* 2006; **17**: 262–270.
39. Coen G, Ballanti P, Balducci A *et al.* Serum osteoprotegerin and renal osteodystrophy. *Nephrol Dial Transplant* 2002; **17**: 233–238.
40. Kurz P, Monier-Faugere MC, Bognar B *et al.* Evidence for abnormal calcium homeostasis in patients with adynamic bone disease. *Kidney Int* 1994; **46**: 855–861.
41. Mallamaci F, Tripepi G, Cutrupi S *et al.* Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. *Kidney Int* 2005; **67**: 2330–2337.
42. Cozzolino M, Brancaccio D, Gallieni M, Slatopolsky E. Pathogenesis of vascular calcification in chronic kidney disease. *Kidney Int* 2005; **68**: 429–436.
43. Victor RG, Haley RW, Willett DL *et al.* The Dallas Heart Study: a population-based probability sample for the multidisciplinary study of ethnic differences in cardiovascular health. *Am J Cardiol* 2004; **93**: 1473–1480.
44. Levey AS, Bosch JP, Lewis JB *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med* 1999; **130**: 461–470.
45. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. *Am J Kidney Dis* 2002; **39**: S46–S103.
46. Agatston AS, Janowitz WR, Hildner FJ *et al.* Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; **15**: 827–832.
47. Bild DE, Bluemke DA, Burke GL *et al.* Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002; **156**: 871–881.
48. Church TS, Levine BD, McGuire DK *et al.* Coronary artery calcium score, risk factors, and incident coronary heart disease events. *Atherosclerosis* 2007; **190**: 224–231.
49. Rumberger JA, Brundage BH, Rader DJ, Kondos G. Electron beam computed tomographic coronary calcium scanning: a review and guidelines for use in asymptomatic persons. *Mayo Clin Proc* 1999; **74**: 243–252.
50. Vliegenthart R, Oudkerk M, Hofman A *et al.* Coronary calcification improves cardiovascular risk prediction in the elderly. *Circulation* 2005; **112**: 572–577.
51. Greenland P, LaBree L, Azen SP *et al.* Coronary artery calcium score combined with framingham score for risk prediction in asymptomatic individuals. *JAMA* 2004; **291**: 210–215.
52. Arad Y, Goodman KJ, Roth M *et al.* Coronary calcification, coronary disease risk factors, C-reactive protein, and atherosclerotic cardiovascular disease events: The St Francis Heart Study. *J Am Coll Cardiol* 2005; **46**: 158–165.
53. Marcovina SM, Albers JJ, Gabel B *et al.* Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). *Clin Chem* 1995; **41**: 246–255.
54. Freedman DS, Otvos JD, Jeyarajah EJ *et al.* Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem* 2004; **50**: 1189–1200.
55. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin Lab* 2002; **48**: 171–180.
56. Ueland T, Bollerslev J, Godang K *et al.* Increased serum osteoprotegerin in disorders characterized by persistent immune activation or glucocorticoid excess-possible role in bone homeostasis. *Eur J Endocrinol* 2001; **145**: 685–690.
57. Khera A, McGuire DK, Murphy SA *et al.* Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol* 2005; **46**: 464–469.